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Taxonomy proposal : To create 6 new species in the genus Cystovirus

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International Committee on Taxonomy of Viruses (ICTV)

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For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document.

Part 1: **TITLE, AUTHORS, etc**

Code assigned:	2017.016B	(to be completed by ICTV officers)
Short title: To create six (6) new species in the genus <i>Cystovirus</i>		
Modules attached (Modules 1, 4 and either 2 or 3 are required.)	1 <input checked="" type="checkbox"/>	2 <input checked="" type="checkbox"/> 3 <input type="checkbox"/> 4 <input checked="" type="checkbox"/>

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List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal)

ICTV Bacterial and Archaeal Viruses Subcommittee

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV:

June 8, 2017

Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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Part 2: **PROPOSED TAXONOMY**

Present the proposed new taxonomy on accompanying spreadsheet

Name of accompanying spreadsheet: 2017.016B.N.v1.Cystovirus_6sp

Please display the taxonomic changes you are proposing on the accompanying spreadsheet module 2017_TP_Template_Excel_module. Submit both this and the spreadsheet to the appropriate ICTV Subcommittee Chair.

Part 4: APPENDIX: supporting material
additional material in support of this proposal

References:

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- Yang Y, Lu S, Shen W, Zhao X, Shen M, Tan Y, Li G, Li M, Wang J, Hu F, Le S (2016) Characterization of the first double-stranded RNA bacteriophage infecting *Pseudomonas aeruginosa*. *Sci Rep* 6:38795.

Annex:

Introduction:

The *Cystoviridae* family currently includes one genus *Cystovirus* and one species, *Pseudomonas virus phi6*. Six additional, cystovirus-related phages with dsRNA genomes have been isolated and completely sequenced (Table 1; Mindich *et al.*, 1999; Hoogstraten *et al.*, 2000; Qiao *et al.*, 2000, 2010; Gottlieb *et al.*, 2002a,b; Mäntynen *et al.*, 2015; Yang *et al.*, 2016).

Species demarcation: We have chosen 95% RNA sequence identity as the criterion for demarcation of species in the *Cystovirus* genus. The members of each of the proposed species differ from those of other species by more than 5% at the RNA level as confirmed with the Clustal Omega Multiple Alignment (Table 2).

Reasons to justify the assignment of the new species to the existing genus *Cystovirus*:

All six new isolates share similar genome and virion organizations with *Pseudomonas* phage phi6. Their genomes are composed of three linear dsRNA segments (S-, M- and L-segments) (Fig. 1) and similar genes can be identified in similar order in each segment (Fig. 1). The genome lengths (12.7 kb – 15.0 kb) and GC contents (53.4–58.8%) are also similar (Table 1). Phylogenetic analyses were done separately for each genome segment using Clustal Omega Multiple Alignment (Fig. 2) and “One Click” at Phylogeny.fr (Fig. 3). Due to the size of the L-segments (over 6000 bp), the phylogenetic analysis was done only for the S- and M-segments with “One Click” (Dereeper *et al.*, 2008). The clustering was different in each tree, suggesting that there has been reassortment of segments between members of the proposed species. Also, exchange of genome segments between the different isolates has been experimentally shown in a few cases (Mindich *et al.*, 1999; Qiao *et al.*, 2000; Qiao *et al.*, 2010).

The virion organization of members of the proposed species, if described, resembles that of members of the type species (Jäälinoja *et al.*, 2007; Wei *et al.*, 2009). Virions are enveloped and their genomes are enclosed in icosahedrally symmetric protein shells.

Table 1. Properties of the proposed members of the *Cystoviridae* family.

Pseudomonas phage	RefSeq No	GenBank accession No	Genome length (kb)	GC%	No. CDS (in positive strand)	% RNA sequence similarity to the type species*
Pseudomonas phage phi6 (member of the type species)	NC_003715; NC_003716; NC_003714	M17461; M17462; M12921	13.4	55.8	13	100
Pseudomonas phage phi8	NC_003299; NC_003300; NC_003301	AF226851; AF226852; AF226853	15	54.5	19	42.4, 44.0, and 44.0 for L, M and S segments
Pseudomonas phage phi12	NC_004173; NC_004175; NC_004174	AF408636; AY039807; AY034425	13.2	55.2	15	43.8, 45.7 and 42.9 for L, M and S segments
Pseudomonas phage phi13	NC_004172; NC_004171; NC_004170	AF261668; AF261667; AF261666	13.7	57.7	13	50.0, 45.6 and 44.5 for L, M and S segments
Pseudomonas phage phi2954	NC_012091; NC_012092; NC_012093	FJ608823; FJ608824; FJ608825	12.7	53.4	14	44.9, 44.2 and 43.6 for L, M and S segments
Pseudomonas phage phiNN		KJ957164; KJ957165; KJ957166	13.3	54.7	13	78.7, 52.1 and 82.8 for L, M and S segments
Pseudomonas phage phiYY		KX074201; KX074202; KX074203	13.5	58.8	14	50.1, 43.7 and 43.2 for L, M and S segments

*Determined using EMBOSS Needle Pairwise Sequence Alignment

Table 2. Nucleotide sequence similarities (%) between the genome segments, determined using Clustal Omega Multiple Alignment. Color code: >95 % = red, >75 % = green, >50 % = yellow.

Nucleotide sequence similarities (%) between the L-segments							
Phage	phi6	phi8	phi12	phi13	phi2954	phiNN	phiYY
phi6	100	38.47	36.42	48.84	36.01	79.46	48.46
phi8	38.47	100	35.8	38.96	34.82	38.51	38
phi12	36.42	35.8	100	35.98	51.13	36.07	35.48
phi13	48.84	38.96	35.98	100	34.9	48.62	62.93
phi2954	36.01	34.82	51.13	34.9	100	35.74	34.76
phiNN	79.46	38.51	36.07	48.62	35.74	100	47.84
phiYY	48.46	38	35.48	62.93	34.76	47.84	100
Nucleotide sequence similarities (%) between the M-segments							
	phi6	phi8	phi12	phi13	phi2954	phiNN	phiYY
phi6	100	38.17	38.99	40.34	39.2	51.18	35.34
phi8	38.17	100	38.47	39.75	38.75	36.48	35.91
phi12	38.99	38.47	100	61.26	37.46	39.15	38.32
phi13	40.34	39.75	61.26	100	37.5	40.66	39.43
phi2954	39.2	38.75	37.46	37.5	100	37.32	34.89
phiNN	51.18	36.48	39.15	40.66	37.32	100	37.25
phiYY	35.34	35.91	38.32	39.43	34.89	37.25	100
Nucleotide sequence similarities (%) between the S-segments							
	phi6	phi8	phi12	phi13	phi2954	phiNN	phiYY
phi6	100	38.41	34.74	40.48	37.39	83.42	40.79
phi8	38.41	100	33.3	37.13	34.02	37.7	37.11
phi12	34.74	33.3	100	33.22	46.43	33.48	38.57
phi13	40.48	37.13	33.22	100	34.45	40.23	37.1
phi2954	37.39	34.02	46.43	34.45	100	36.41	41.64
phiNN	83.42	37.7	33.48	40.23	36.41	100	40.18
phiYY	40.79	37.11	38.57	37.1	41.64	40.18	100

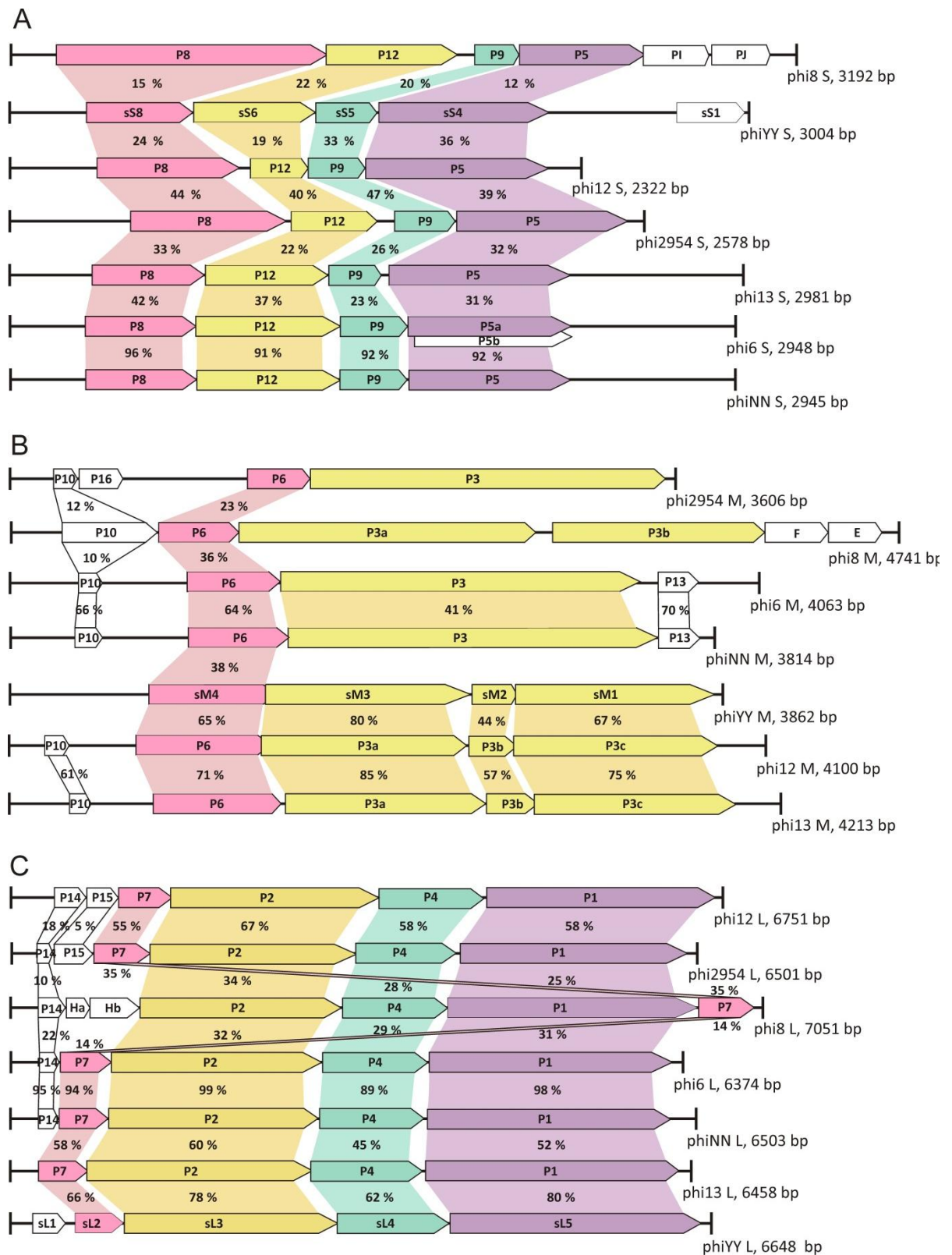


Fig. 1. Genome maps of the segments S (A), M (B) and L (C) of the proposed members of the *Cystoviridae*. Open reading frames (ORFs) of the predicted positive strands are depicted and amino acid sequence similarities (%) between corresponding ORFs are indicated. Comparisons were conducted with EMBOSS Needle Pairwise Sequence Alignment. The order of the genome segments follows the clustering in the phylogenetic trees presented in Fig. 2.

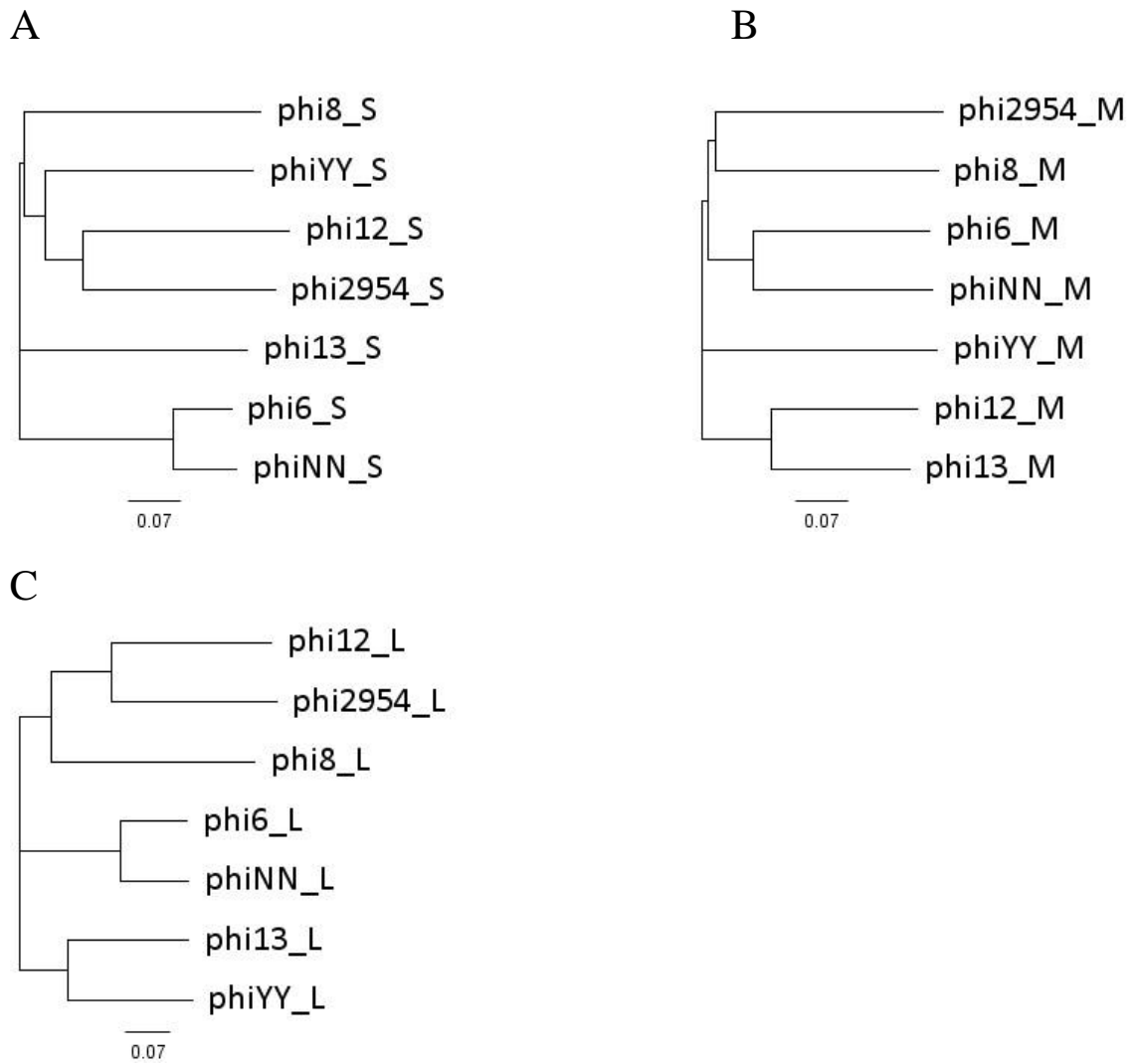


Fig. 2. Phylogenetic trees showing relationships between proposed members of the *Cystoviridae* based on nucleotide sequence comparisons of the segments S (A), M (B) and L (C), constructed using Clustal Omega Multiple Alignment.

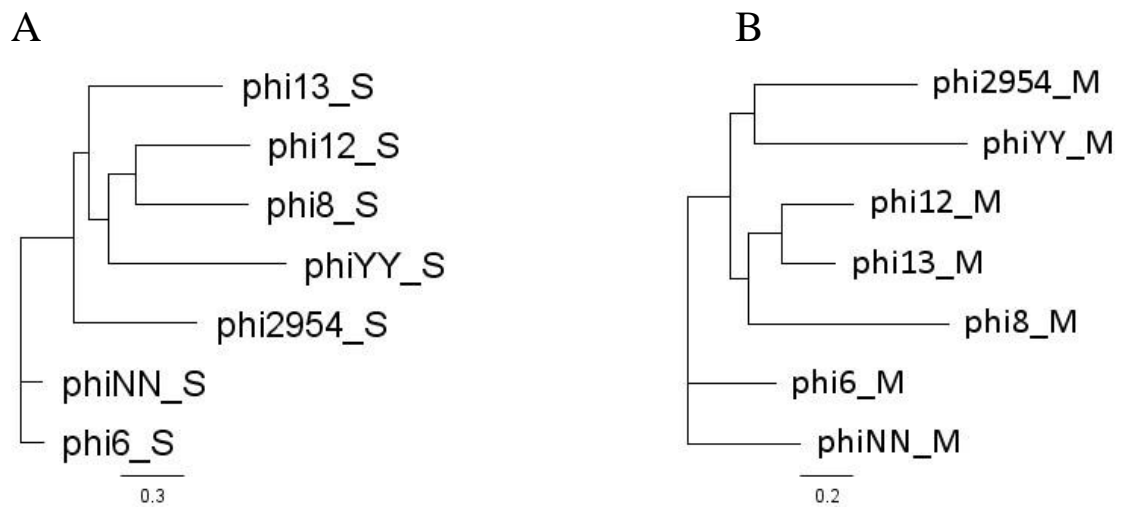


Fig. 3. Phylogenetic trees showing relationships between proposed members of the *Cystoviridae* based on nucleotide sequence comparisons of the segments S (A), M (B) and L (C), constructed using “One Click” at Phylogeny.fr.